Speciation of Inorganic Arsenic in Baby Rice Cereals Using HPLC-ICP-MS

Application Note

Food Safety

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Abstract

A survey of baby rice cereals using ICP-MS and the method specified in FDA EAM: Section 4.11 detected all arsenic species in all of the cereals tested. Limit of Detection (LOD) values for As species ranged from approximately 0.9 ng/g to 1.8 ng/g, and Limit of Quantitation (LOQ) from 7 µg/kg to 14 µg/kg, and %RSDs ranged from 1.7 to 6.7%.

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Introduction

Rice is one of the main sources of inorganic arsenic (iAs) consumed in the world population's diet [1]. Arsenic occurs naturally in the environment and can also be present as the result of human activity, such as introduction to the soil through the use of arsenic-based pesticides prior to the 1970s. Rice plants are especially efficient at acuminating arsenic from their environment, because the flooded areas in which they are grown make it easier to take up arsenic compounds [2,3].

Inorganic arsenic is a known carcinogen, and chronic exposure to low levels of arsenic has been linked to increased risk of bladder, lung, and skin cancer, as well as Type 2 diabetes and cardiovascular disease [4]. Brown rice tends to contain more arsenic than white rice [1,5,6]. Rice samples from the United States have shown higher inorganic arsenic levels compared to other samples from around the world, and infant rice cereal sold in the US is generally made from American rice [2].

This application note describes use of the high performance liquid chromatography/inductively coupled plasma mass spectrometry (HPLC-ICP-MS) method specified in the FDA Elemental Analysis Manual (EAM): Section 4.11 for the speciation of arsenic in baby rice cereals. The arsenic species are separated using isocratic anion-exchange HPLC, and the ICP-MS is used as an arsenic-specific detector monitoring *m/z* 75 for arsenic-containing chromatographic peaks. It is operated in helium collision cell mode to eliminate interference from possible coeluting chloride species. Limits of detection and quantitation (LOD and LOQ) were below 15 µg/g for all arsenic species, with relative standard deviations (%RSDs) all below 7%. The method has been used to provide information on arsenic levels in rice and rice-based infant cereals necessary to help understand the health risk to infants [7].

Experimental

Chemicals and Standards

All solutions were prepared using deionized water with resistance > 18 MΩ•cm with a Milli-Q system (Millipore).

Chemical/Standard	Source
Nitric acid, OPTIMA ultra-pure grade	Fisher Scientific
Hydrogen peroxide, OPTIMA ultra-pure grade	Fisher Scientific
Ammonium phosphate	ACROS
Ammonium hydroxide, ultrapure	Fisher Scientific
Isopropanol, HPLC-grade	Fisher Scientific
Arsenite Stock Standard (AsIII), 1,000 mg/L	Spex Certiprep
Arsenate Stock Standard (AsV), 1,000 mg/L	Spex Certiprep
Monosodium acid methane arsonate (MMA, 98.5% purity)	Chem Service
Disodium acid methane arsonate (DMA, 98.9% purity)	Chem Service
Arsenobetaine (AsB)	Fluka
Rhodium/germanium internal standard	Agilent Technologies
ICP-MS Arsenic Standard	Plasma

Table 1. Rice Flour Standard Reference Materials

SRM/CRM	Total As (µg/kg)	As(III) (µg/kg)	As(V) (µg/kg)	iAs (µg/kg)	DMA (µg/kg)	MMA (µg/kg)
1568a	290 ± 30*	60 ± 12**	39 ± 8**	100 ± 20**	171 ± 34**	11 ± 2**
1568	410 ± 50*	85 ± 17**	31 ± 6**	116 ± 23**	285 ± 57**	22 ± 4**

* Certified Value with Uncertainty expressed as a 95% Confidence Interval or 95% Confidence Interval plus an allowance for systematic error.

** Uncertainty expressed as ± 20% of the average value from the best available data.

Standard reference materials (SRMs)

NIST (National Institute of Standards and Technology) Standard Reference Material (SRM) 1568a and 1568 rice flour were used as quality control materials for both As speciation measurements and total As determinations (Table 1). The certified values for NIST 1568, for total arsenic are 410 ± 30 µg/kg and the certified values for As(III), DMA, and As(V) in NIST 1568 are 85 ± 17, 285 ± 57, and 31 ± 6 µg/kg respectively.

Instruments

Speciation analysis was done using an Agilent 1200 Infinity LC system consisting of a vacuum degassing unit, binary pump, autosampler, and a 10-port valve (two ports were plugged). An external pump was used (LC-20AD, Shimadzu) for the internal standard spike introduction .The HPLC system was connected to the Agilent 7700x ICP-MS using 1/16 × .0025 × 12 inch Peek tubing (Upchurch Scientific) and equipped with a MicroMist nebulizer and Scott double pass spray chamber. The instrument run conditions are listed in Table 2.

Standard solutions

All standard solutions were prepared daily through serial dilution of stock species standards according to the FDA method (EAM 4.11). All arsenic stock standard solutions (As(III), DMA, MMA, As(V)) were prepared in water at 1,000 µg/mL. The arsenic concentrations of the DMA and MMA standards were verified using ICP-MS analysis. The total arsenic concentrations of 1 µg/g MMA and DMA standards were determined using a calibration curve of ICP-MS Arsenic Standard. These concentrations were used to recalculate the stock standard concentrations and the new values were then applied to the future calculations.

Working standards were then diluted with mobile phase to desired concentrations using the 200 µg/mL stock solution. All standard solutions were stored in the dark at 4 °C. On the morning of the experiment, a fresh mixed standard solution was made. The internal standard for the speciation method was 2 ng/g of As(V) in mobile phase, and it was injected post-column to monitor and to correct for signal drift. The internal standard for the totals method was rhodium/germanium with 18% isopropanol (ICP-MS Internal Standard Mix 100 mg/L, p/n 5188-6525).

Table 2. HPLC and ICP-MS Run Conditions

HPLC conditions	
Guard column	Hamilton (p/n 79446)
Column	Hamilton PRP-X 100 anion exchange, 4.1 × 250, stainless steel, 10 µm
Mobile phase	10 mM ammonium phosphate pH 8.25, isocratic run
Flow rate	1 mL/min
Injection volume	100 µL
ICP-MS conditions	
RF power	1,500 W
Plasma gas flow	15 L/min
Dilution gas flow	0.17 L/min
Nebulizer gas flow	1.1 L/min
Nebulizer type	MicroMist
Sampling depth	8.5 mm
Spray chamber temperature	2 °C
Collision cell gas	He, 4.3 mL/min
Data acquisition mode	Time-resolved analysis: *m/z* 75 for $^{75}As^+$ and *m/z* 77 for $^{40}Ar^{35}Cl^+$
Dwell times	0.8 s for *m/z* 75; 0.2 s for *m/z* 77

The standard for LOD and LOQ determination was prepared at 0.2 ng/g each of As(III), DMA, MMA, and As(V) in mobile phase. A resolution check standard was prepared using 5 ng/g As(III) and Arsenobetaine (AsB) in mobile phase, and it was used to determine the separation of AsB and As(III). A new resolution check standard was prepared when approximately 50% of As(III) had been converted to As(V).

Samples

Baby rice cereals were purchased at different locations and various grocery stores in the following US states; Illinois, California, Texas, and North Dakota. The 31 infant rice cereals were produced by seven different manufacturers. Table 3 lists all the information that could be obtained from the baby rice cereals containers.

Table 3. Summary of the Infant Rice Cereals Tested

Brand	Sample ID	Product description	Ingredients	Location of purchase
A	Baby_1	Mixed grain cereal	Whole wheat flour, rice whole grain oat flour, soybean oil	IL
A	Baby_2	Organic brown rice	Organic whole grain brown rice flour, soy lecithin	IL
B	Baby_3	Organic whole grain rice cereal	Organic whole grain brown rice, tocopherols (vitamin E), electrolytic iron	IL
C	Baby_4	Organic rice cereal	Organic whole grain brown rice flour, sunflower lecithin, electrolytic iron	IL
A	Baby_5	Rice single grain cereal	Organic whole grain brown rice flour, sunflower lecithin, electrolytic iron	IL
C	Baby_6	Organic rice cereal	Organic whole grain brown rice flour, sunflower lecithin, electrolytic iron	TX
D	Baby_7	Rice cereal, single grain	Rice flour, soybean oil, soy lecithin	CA
E	Baby_8	Rice cereal	Rice flour, soybean oil, soy lecithin	ND
A	Baby_9	Organic brown rice cereal	Organic whole grain brown rice, tocopherols(vit E), electrolytic iron	ND
B	Baby_10	Organic brown rice cereal	Organic whole grain brown rice, tocopherols (vit E), electrolytic iron	ND
B	Baby_11	Organic brown rice cereal	Organic whole grain brown rice, tocopherols (vit E), electrolytic iron	CA
A	Baby_12	Organic brown rice cereal	Organic brown rice cereal	TX
F	Baby_13	Rice cereal	Rice flour, sunflower lecithin	ND
F	Baby_14	Rice cereal	Rice flour, sunflower lecithin	CA
F	Baby_15	Rice cereal	Rice flour, sunflower lecithin	CA
A	Baby_16	Rice cereal	Rice flour	TX
F	Baby_17	Rice cereal	Rice flour, sunflower lecithin	TX
A	Baby_18	Mixed grain cereal	Whole wheat flour, rice whole grain, oat flour, soybean oil	CA
A	Baby_19	Mixed grain cereal	Whole wheat flour, rice whole grain, oat flour, soybean oil	TX
A	Baby_20	Mixed grain cereal	Whole wheat flour, rice whole grain, oat flour, soybean oil	ND
A	Baby_21	Mixed grain cereal	Whole wheat flour, rice whole grain, oat flour, soybean oil	CA
A	Baby_22	Mixed grain cereal	Whole wheat flour, rice whole grain, oat flour, soybean oil	ND
A	Baby_23	Mixed grain cereal	Whole wheat flour, rice whole grain, oat flour, soybean oil	CA
G	Baby_24	Organic brown rice cereal	Organic whole grain brown rice flour, sunflower lecithin	CA
G	Baby_25	Organic brown rice cereal	Organic whole grain brown rice flour, sunflower lecithin	CA
A	Baby_26	Rice single grain cereal	Rice flour, tri- and dicalcium phosphate, soybean oil, soy lecitin, tocopherols, vitamins A and B, B group	CA
A	Baby_27	Mixed grain cereal	Whole wheat flour, whole grain oat flour, tri- and dicalcium phosphate, soybean oil, soy lecitin, tocopherols, vitamins A and B, B group	CA
B	Baby_28	Organic whole grain rice cereal with apples	Organic whole grain brown rice flour, organic apple puree, vitamin E, iron, and others	CA
B	Baby_29	Organic whole grain rice cereal	Organic whole grain brown rice, vitamin E, iron	CA
B	Baby_30	Organic whole grain rice cereal	Organic whole grain brown rice, vitamin E, iron	CA
A	Baby_31	DHA and probiotic single grain rice cereal	Rice flour, vitamins, minerals, soybean oil, soy lecitin, tocopherols, tuna oil	CA

Sample preparation

All baby rice cereals were analyzed for total arsenic and arsenic speciation. Infant cereals were analyzed directly from the container and were not further dried or homogenized. For the total arsenic analysis, the same hot block digestion method was used, but the samples were then injected directly into the ICP-MS without HPLC separation.

The hot block digestion method [3] was used for both the total and speciation analysis. A gravimetric method was applied to all preparations in this study. One gram of infant rice cereal sample was weighed precisely into a pre-weighed 50 mL polypropylene centrifuge tube (with lid). To this tube was added 10 g of 0.28 M HNO_3. and the sample was vortexed for 10–30 seconds. The tightly capped tube was then placed in a preheated block digestion system at 95 °C for 90 minutes.

After the sample was cooled, 6.7 g of deionized water was added. and the rice cereal suspension was centrifuged at 3500 rpm for 10 minutes. The supernatant was passed through a 0.45 µm nylon syringe filter attached to a 3 mL disposable syringe. Approximately the first 1 mL was discarded through the filter to waste, and 1 g of filtrate was then transferred to a 15 mL tared centrifuge tube. To this was added 2 g of pH Adjustment Solution which was prepared by adding ammonium hydroxide to the mobile phase to a pH of 9.95 ± 0.05.

The pH of the final preparation of the method blank and samples was adjusted to between 6 and 8.5 with the pH Adjustment Solution. A portion of the resulting sample solution was transferred to a polypropylene autosampler vial for analysis by HPLC-ICP-MS

Results and Discussion

Chromatographic Separation

Figure 1 shows a typical separation. Blank tests were performed to investigate possible arsenic contamination; none was detected.

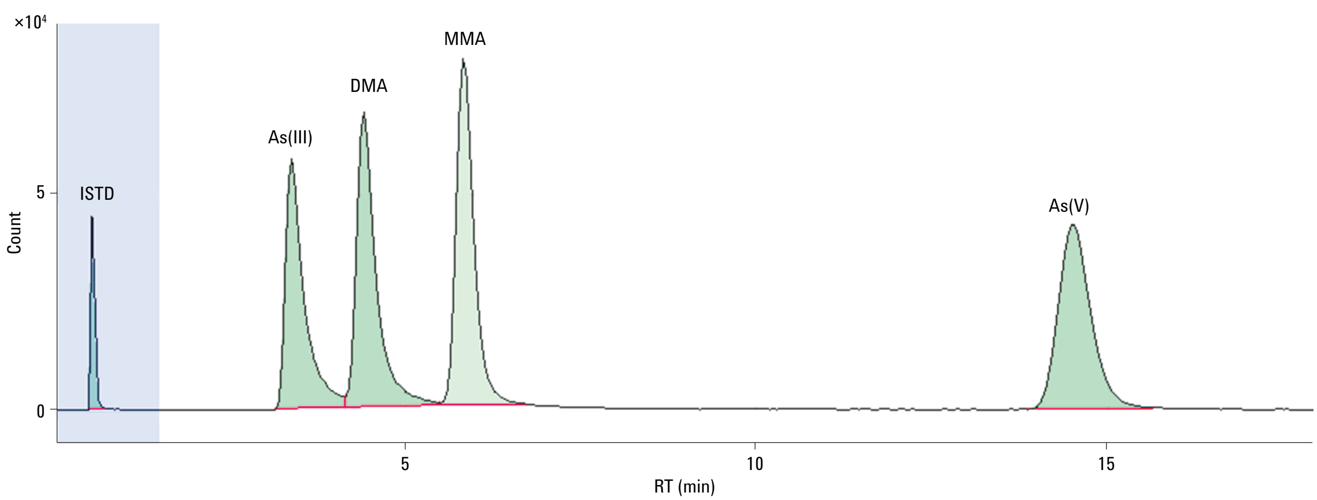

Figure 1. Chromatographic separation of arsenic standards at 5 ng/g.

Method precision

To validate the method for arsenic speciation and total methods, the NIST SRM 1568 rice flour was analyzed. Results for speciated and total As in the certified reference material were in good agreement with certified values. The repeatability of the EAM 4.11 method for analysis of baby rice cereals was determined using a set of SRM replicates (Table 4). Repeatability (%RSD) was 1.74% for total arsenic by the As speciation method, 5.3% for the total arsenic by As total method, and 2.8% for iAs.

Calibration curves

The calibration curves for arsenic species were determined using As(III), DMA, MMA, and As(V) standards with concentrations of 0.25, 0.4, 1.0, 5.0 and 20 ng/g (Figure 2). The standard curves were linear for this range with a coefficient of variation (R^2) of 1.00. Recovery for the calibration check standard, which was composed of 10 ng/g each of As(III), DMA, MMA, and As(V), was 99–113% with % RSD < 4%.

Table 4. Method Precision

Sample name (separate preparations)	**As(III) (µg/kg)**	**DMA (µg/kg)**	**MMA (µg/kg)**	**As(V) (µg/kg)**	**iAs[a] (µg/kg)**	**Total As (by speciation method)**	**Total As (by totals method)**	**Mass balance[b]**
Certified value	85 ± 17	285 ± 57	22 ± 4	31 ± 6	116 ± 23	410 ± 50	410 ± 50	
1568_rep1	65	289.9	24.8	40.6	105.6	420.3	398.4	105.5
1568_rep2	68.5	289	24.6	42.5	110.9	424.5	445.1	95.4
1568_rep3	66.9	300.6	25	44.7	111.6	437.2	405.2	107.9
1568_rep4	70.3	288.7	21.6	37.5	107.8	418.1	442	94.6
1568_rep5	69.8	289.4	22.8	43.3	113.1	425.4	442.3	96.2
Average	68.1	291.5	23.8	41.7	109.8	425.1	426.6	99.9
Standard deviation (SD)	2.2	5.1	1.5	2.8	3.1	7.4	22.8	6.3
%RSD	3.2	1.7	6.3	6.7	2.8	1.7	5.3	6.3

[a] iAs equals the sum of As(III) and As(V).

[b] Mass balance = Total As from speciation (As(III) + DMA + MMA + As(V)) /(total As from total As method)

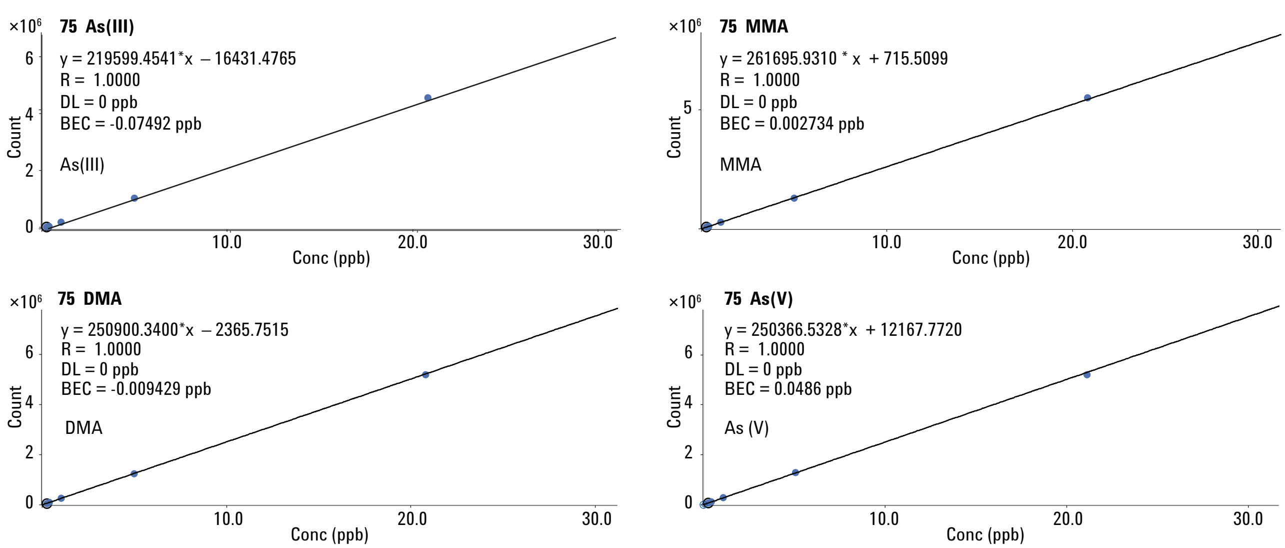

Figure 2. Calibration curves for the EAM 4:11 method.

Limits of detection and quantitation

The Analytical Solution Detection Limit (ASDL), Analytical Solution Quantitation Limit (ASQL), and method Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined for the arsenic speciation analysis in rice using the HPLC-ICP-MS method (Table 5). The LOD values for As species ranged from approximately 0.9 ng/g to 1.8 ng/g, and LOQ from 7 µg/kg to 14 µg/kg .

Table 5. ASDL, ASQL, LOD and LOQ of Arsenic Speciation Analysis by HPLC-ICP-MS

	As(III)	DMA	MMA	As (V)
ASDL (ng/g)	0.034	0.017	0.024	0.035
LOD (µ/kg)	**1.68**	**0.87**	**1.18**	**1.74**
ASQL (ng/g)	0.262	0.135	0.184	0.273
LOQ (µ/kg)	**13.1**	**6.8**	**9.2**	**13.6**

Recoveries

Generally, spiked sample recoveries for iAs, DMA and MMA were excellent, considering arsenic conversion from As(III) to As (V). The recoveries for iAs, DMA, and MMA ranged from a low of 97.3% for iAs to a high of 115.0% for DMA (Table 6).

Table 6. % Recovery of Spiked Arsenic (n = 2)

Spike concentration of each species	As(III)	DMA	MMA	As(V)	iAs
50 ng/g	104.1 ± 38.2	115.0 ± 1.2	104.8 ± 1.1	109.1 ± 38.3	106.5 ± 0.6
75 ng/g	85.1 ± 1.3	108.6 ± 2.9	106.8 ± 0.8	116.4 ± 0.9	100.5 ± 1.1
100 ng/g	67.8 ± 32.8	112.4 ± 2.2	105.0 ± 0.8	143.7 ± 28.5	105.2 ± 2.6
150 ng/g	74.7 ± 10.1	104.0 ± 9.4	106.0 ± 6.1	120.7 ± 7.7	97.3 ± 8.9
150 ng/g	67.9 ± 15.6	112.9 ± 2.8	106.6 ± 0.5	142.8 ± 12.4	104.8 ± 1.8
225 ng/g	88.1 ± 1.5	109.3 ± 1.2	105.2 ± 1.0	118.2 ± 3.0	102.9 ± 2.2

Total and speciation arsenic concentrations In baby rice cereals

Table 7 shows the total and speciation results for arsenic in baby rice cereals. There was no significant difference between the two duplicates run on each cereal sample. All 31 of the cereals tested contained arsenic, as well as inorganic arsenic (iAs), which is defined as the sum of the As(II) and As(V) concentrations. The average total arsenic and iAs concentrations were 174.4 and 101.4 µg/kg, respectively in rice cereals. The average percentage of iAs as a component of the average total arsenic in the baby rice cereals tested was 58.2%.

The mass balance between the As speciation and totals methods (sum of all As species by the speciation method, divided by the As concentration from the totals method) ranged from 85.8% to 106.0%, with an average mass balance of 96.7%. All mass balances are shown in Table 7.

The average total arsenic and iAs concentrations were lower in mixed grain cereals (105.1 and 62.8 µg/kg, respectively). The average percentage of iAs of as a component of the average total arsenic of the average total arsenic in the baby mixed grain cereals tested was thus 59.8%.

As listed in Table 3, all 31 baby rice cereals were made from the flour of either organic rice, organic rice whole grain, mixed grain (whole wheat, rice whole grain, and oat), or rice. Results show that levels of inorganic arsenic (iAs) varied greatly across all rice cereals (Table 7). There was no substantial difference in iAs levels between organic and conventional rice cereals (an average of 98 ng/g in organic white rice cereal versus 101 ng/g in conventional white rice cereal).

In the baby rice cereals tested, the total arsenic concentration ranged from 79.9 to 277 ng/g. The iAs, as a percent of total arsenic, ranged from 23 to 81%. The major organic arsenic species detected was DMA (Figure 3). MMA was either not detected or only trace levels were found (Table 7).

Table 7. Results from the 23 Infant Rice Cereals Analyzed in this Application Note

Sample ID	As(III) (µg/kg)	DMA (µg/kg)	MMA (µg/kg)	As(V) (µg/kg)	iAs[a] (µg/kg)	Total As (by speciation) (µg/kg)	Total As (by totals) (µg/kg)	% Mass balance[b]	Inorganic As per serving (µg)
Mixed grain cereal_1	20.6 ± 7.7	27.5 ± 0.2	TR (1.4)	36.6 ± 8.4	57.2 ± 0.7	86.2 ± 0.5	88.3 ± 1.3	97.6	0.9
Mixed grain cereal_18	18.1 ± 3.3	35.2 ± 1.4	TR (1.9)	44.9 ± 4.1	63.0 ± 0.7	100. ± 2.0	112. ± 5.7	89.1	0.9
Mixed grain cereal 19	12.1 ± 9.2	23.0 ± 0.7	TR (1.5)	43.3 ± 7.9	55.5 ± 1.3	79.9 ± 1.4	88.0 ± 1.4	90.9	0.8
Mixed grain cereal 20	4.84 ± 1.3	50.5 ± 0.6	TR (2.3)	58.9 ± 1.1	63.7 ± 0.1	116. ± 0.6	124. ± 0.5	93.8	1.0
Mixed grain cereal_21	13.0 ± 2.1	48.2 ± 0.4	TR (2.4)	58.5 ± 2.0	71.6 ± 0.1	122. ± 0.5	126. ± 0.4	96.4	1.1
Mixed grain cereal_22	4.83 ± 0.5	50.0 ± 1.7	TR (2.5)	64.0 ± 1.1	68.8 ± 0.6	121. ± 2.7	131. ± 9.1	92.2	1.0
Mixed grain cereal _23	9.89 ± 9.6	49.1 ± 0.6	TR (3.0)	55.4 ± 9.1	65.3 ± 0.4	117. ± 0.7	128. ± 4.6	91.5	1.0
Mixed grain cereal _27	19.3 ± 0.2	38.1 ± 0.5	TR (1.3)	37.7 ± 5.3	57.1 ± 5.0	96.5 ± 3.9	109. ± 0.4	88.1	0.9
Organic, rice cereal_4	37.8 ± 8.6	153. ± 0.6	TR (8.9)	67.3 ± 4.9	105. ± 3.7	267. ± 3.8	272. ± 7.9	98.2	1.6
Organic, rice cereal_6	26.0 ± 0.4	51.8 ± 1.5	TR (2.8)	64.7 ± 1.9	90.7 ± 1.4	145. ± 3.1	146. ± 7.3	99.3	1.4
Rice single grain cereal_5	65.7 ± 5.7	100. ± 0.1	TR (4.9)	72.0 ± 3.7	137. ± 1.9	243. ± 2.5	230. ± 10.	106	2.1
Rice single grain cereal_7	32.9 ± 0.8	89.2 ± 0.9	TR (5.1)	85.9 ± 0.3	118. ± 1.1	213. ± 0.7	212. ± 8.5	100	1.8
Rice single grain cereal_8	56.6 ± 1.8	126. ± 1.0	TR (6.6)	76.1 ± 0.5	132. ± 1.3	265. ± 0.8	256. ± 0.4	104	2.0
Rice single grain cereal_13	27.6 ± 0.1	35.3 ± 0.0	TR (1.5)	45.2 ± 1.7	72.8 ± 1.6	109. ± 1.2	108. ± 1.7	101	1.1
Rice single grain cereal_14	24.7 ± 1.0	32.2 ± 0.9	TR (3.0)	43.5 ± 0.0	68.2 ± 0.9	103. ± 1.8	101. ± 1.7	102	1.0
Rice single grain cereal_15	24.1 ± 1.6	30.0 ± 0.3	TR (1.6)	33.8 ± 0.0	58.0 ± 1.7	89.7 ± 3.1	92.5 ± 3.2	97.1	0.9
Rice single grain cereal_16	59.9 ± 22.	107. ± 1.5	TR (3.4)	65.6 ± 14.	125. ± 8.1	236. ± 9.6	227. ± 1.6	104	1.9
Rice single grain cereal_17	35.2 ± 10.	34.3 ± 0.9	TR (1.8)	54.7 ± 7.0	90.0 ± 3.1	126. ± 3.1	127. ± 1.4	99.2	1.4
Rice single grain cereal_26	60.1 ± 8.3	97.1 ± 1.8	TR (2.7)	47.4 ± 8.2	107. ± 0.1	207. ± 2.1	241. ± 2.4	85.8	1.6
Rice single grain cereal_31	58.6 ± 3.7	104. ± 1.6	TR (2.8)	39.1 ± 2.7	97.8 ± 0.9	205. ± 3.5	237. ± 0.9	86.4	1.5
Organic, whole grain rice cereal_3	16.2 ± 7.3	15.9 ± 0.5	0.0	51.7 ± 5.4	68.0 ± 1.9	84.4 ± 3.0	85.1 ± 1.7	99.1	1.0
Organic brown rice cereal_2	21.7 ± 0.2	42.9 ± 0.1	TR (4.2)	111. ± 1.2	133. ± 1.0	180. ± 0.2	178. ± 0.1	101	2.0
Organic brown rice cereal_9	21.7 ± 10.	27.7 ± 1.0	0.0	34.3 ± 9.1	56.0 ± 1.7	84.7 ± 2.6	83.9 ± 2.6	101	0.8
Organic brown rice cereal_10	24.2 ± 1.3	206. ± 4.2	TR (6.5)	39.5 ± 2.6	63.8 ± 4.0	277. ± 8.0	261. ± 7.0	106	1.0
Organic brown rice cereal_11	21.3 ± 3.1	38.5 ± 0.3	TR (2.8)	107. ± 0.3	128. ± 2.7	169. ± 2.0	165. ± 7.8	103	1.9
Organic brown rice cereal_12	23.0 ± 3.5	107. ± 1.0	TR (3.4)	65.7 ± 1.3	88.8 ± 2.2	200. ± 2.9	206. ± 13.	97.2	1.3
Organic brown rice cereal_24	18.0 ± 0.9	25.8 ± 0.0	TR (1.7)	69.4 ± 0.7	87.4 ± 1.6	114. ± 2.0	127. ± 1.9	90.2	1.3
Oganic brown rice cereal_25	23.1 ± 1.8	27.6 ± 0.0	TR (2.0)	73.0 ± 0.6	96.2 ± 2.4	125. ± 1.9	133. ± 0.0	94.2	1.4
Organic, whole grain rice cereal with apples_28	41.8 ± 1.2	32.2 ± 0.0	TR (1.5)	63.3 ± 0.4	105. ± 0.7	139. ± 0.9	160. ± 1.0	86.6	1.6
Organic, whole grain rice cereal_29	51.5 ± 2.2	65.0 ± 0.3	TR (2.6)	89.6 ± 1.2	141. ± 0.9	208. ± 0.1	242. ± 4.4	86.0	2.1
Organic, whole grain rice cereal_30	81.7 ± 7.8	51.8 ± 0.6	TR (1.2)	76.7 ± 13.	158. ± 6.0	211. ± 5.4	244. ± 0.0	86.4	2.4

[a] iAs equals the sum of As(III) and As(V)

[b] Mass balance: Total As from speciation (As(III) + DMA + MMA + As(V)) / total As from totals method; all samples were analyzed in duplicate (n = 2).

TR = trace level

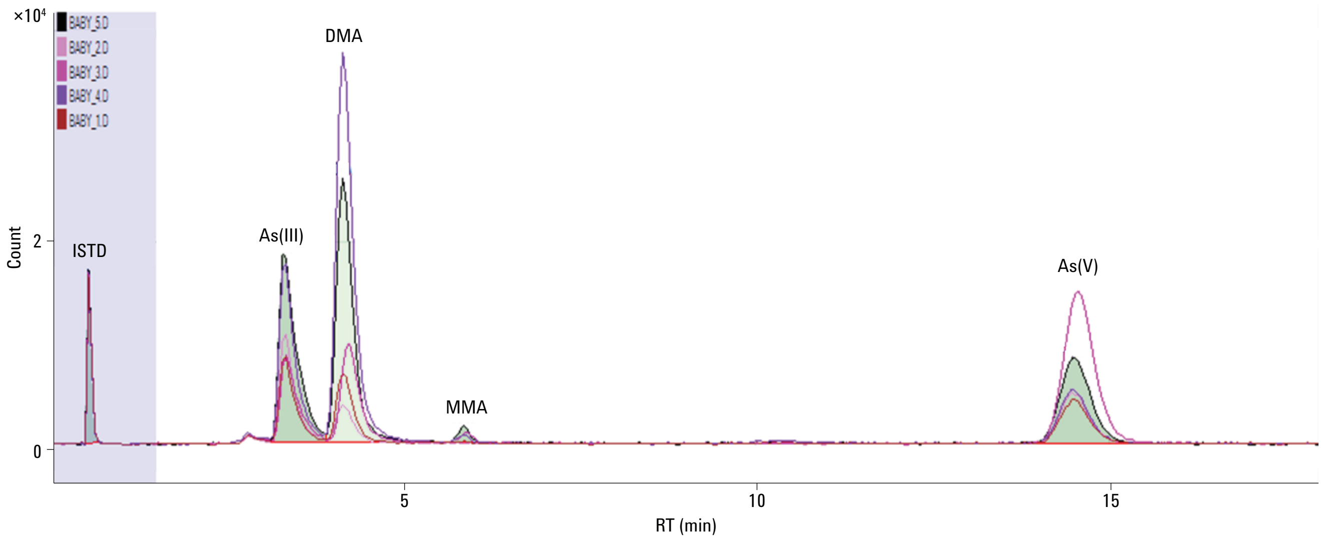

Figure 3. A representative chromatogram overlay of arsenic speciation in five infant rice cereals.

The mixed grain rice cereal contained the least total (105 ng/g average value) and inorganic arsenic (63 ng/g average value), by speciation.

The results of the analysis of the baby rice cereals were compared with results obtained in the literature [1,8]. Concentrations of total arsenic and inorganic arsenic were not as high as have been reported for some of the infant and toddler formulas. This is due to the fact that none of the baby rice cereals in this study contained organic brown rice syrup (OBRS). It has been reported that toddler formulas containing OBRS as the primary ingredient can contain arsenic levels over 20 times higher than those in non-OBRS formulas [1].

Comparison to FDA findings

On September 19, 2012, the FDA released the first analytical results of nearly 200 samples of rice and rice products collected in the U.S. marketplace. Among these samples, 32 were rice cereal samples and only 3 were infant rice cereal samples (Table 8). The FDA reported iAs values were much higher than our results. Average inorganic arsenic (iAs) was 3.5 µg/serving in FDA tested rice cereals (range of 1.5–9.7 µg/serving), and 2.7 µg/serving in infant rice cereals.

The 23 infant rice cereal samples that were analyzed in this study had an average 1.5 µg of iAs per serving, and eight mixed grain cereals contained 0.9 µg/serving iAs on average. The iAs values ranged from 0.8 to 2.4 µg/serving (Table 7).

Table 8. FDA Summary Analytical Results for Infant Rice Cereals, from the "Rice/Rice Product Sampling - September 2012"

Sample ID	Product category	Sample description	Country of origin	Inorganic As per serving (µg/serving*)
70145	Rice Cereal	Organic whole grain rice cereal (infant)	NR	3.2
720341	Rice Cereal	Organic whole grain rice cereal (infant)	NR	2.9
719983	Rice Cereal	Rice single grain (infant)	NR	2.0

*Serving size based on Reference Amount Customarily Consumed (RACC) per 21CFR 101.12 for infant rice cereals, which used a value of 15 g to calculate the µg of inorganic arsenic per serving.

Conclusions

This study demonstrated the successful application of the HPLC-ICP-MS method specified in the FDA Elemental Analysis Manual (EAM): Section 4.11. for the determination of arsenic species in infant rice cereals. The sensitivity (LODs and LOQs) and precision (%RSD) were sufficient to detect low levels of all arsenic species, including iAs levels that were below those previously found in baby rice cereals in an FDA study. This study provides new and much needed information for the assessment of arsenic levels in rice and rice-based infant cereals.

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